Basophilic (Mucoid) Degeneration of Myocardium

A Disorder of Glycogen Metabolism

Juan Rosai, MD and Eduardo F. Lascano, MD

A RATHER COMMON, albeit frequently ignored, finding in hematoxylin-eosin stained sections of human myocardium is the presence of a basophilic, finely granular material in the cytoplasm of isolated myocardial fibers. This condition, first described by Geipel [1] in 1905, has been variously called basophilic degeneration (BD),[2] mucoid degeneration,[3] mucinous degeneration,[4] or cardiac colloid.[5] Despite the fact that a good number of studies have been made on this peculiar condition,[1-14] very little is known of its significance, mechanism of formation and chemical composition. Manion [13] has recently summarized the present, rather unsatisfactory situation regarding the knowledge of BD.

In an effort to determine the chemical nature of BD and from there gain insight into the events leading to its formation, we have performed the following histochemical, fine structural and biochemical studies.

Material and Methods

Histochemistry

Sections of myocardium obtained from the left ventricle were examined microscopically in 135 consecutive necropsies. The age of the individuals ranged from newborn to 92 years. The tissues were fixed in 10% formalin, embedded in paraffin and stained with hematoxylin-eosin and periodic acid-Schiff reaction (PAS).

Five cases with extensive BD were selected from this group, examined for autofluorescence and stained with the following: Langhans' iodine, Best's carmine, Gomori's lithium-silver, Feulgen reaction, Mallory's phosphotungstic acid-hematoxylin, Bennhold's Congo red, crystal violet, Thioflavine T, Perls', Sudan IV, von Kossa, Verhoeff's elastin, Mayer's mucicarmine, Lillie's azur A-eosin B, toluidine blue, Alcian blue at pH 2.5 and 1.0, Mowry's colloidal iron, and Millon reaction.

Similar sections were stained with PAS after treatment with (1) 1% malt diastase in pH 6.0 phosphate buffer at 37 C for 30 min, 8 hr and 48 hr; (2) 100% aqueous chloral hydrate, overnight, at room temperature, followed by diastase for 30 min; (3) boiling of formalin-fixed myocardium in distilled water for 1 hr, then embedding with paraffin and digestion of the sections by diastase for 30 min; (4) 1% amyloglucosidase (glucoamylase or α-1,4-glucan glucohydrolase [from Rhiszo-

From the Department of Pathology, Washington University School of Medicine and John Cochran Veterans Administration Hospital, St. Louis, Missouri; and the Department of Pathology, Hospital Regional, Mar del Plata, Argentina.

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Address for reprint request: Dr. Juan Rosai, Division of Surgical Pathology, Barnes Hospital, Barnes Hospital Plaza, St. Louis, Mo 63110.

pus genus mold, Sigma]) in pH 4.5 acetate buffer at 37 C for 1 hr; (5) 0.4% pectinase in pH 4.0 acetate buffer at 37 C for 48 hr; (6) 0.1% testicular hyaluronidase in pH 5.5 phosphate buffer at 37 C for 3 hr; (7) 0.01% trypsin in pH 8.9 phosphate buffer at 37 C for 1 hr; and (8) xylene, chloroform and ethyl ether, sequentially, 24 hr each.

Similar sections were stained with Alcian blue (pH 2.5) after treatment with (1) 1% malt diastase for 30 min; (2) 100% aqueous chloral hydrate, overnight; (3) boiling of formalin-fixed myocardium in distilled water for 1 hr. The same reactions were performed with myocardium from a previously reported case of glycogenosis IV [15]; and with nomal human liver, stomach, large bowel and uterine cervix.

Electron Microscopy

A fine structural study was carried out in blocks of myocardium of 2 necropsy cases, which were known by light microscopy to contain extensive deposits of BD. One of these 2 patients had severe hypothyroidism. The tissues, which had been fixed in buffered 10% formalin, were osmicated, embedded in epoxy and sectioned with a Reichert automatic ultramicrotome. The sections were stained sequentially with uranyl acetate and lead citrate and examined with a Philips EM 200 electron microscope.

Electron microscopic examination was also performed in tissue from the same cases after the following treatments: (1) digestion of the thin sections with 0.5% malt diastase in pH 5.0 phosphate buffer at 37 C for 18 hr, previous oxidation with 10% periodic acid for 30 min [16]; (2) digestion of the formalin-fixed block (± 1 cu mm) with 1% malt diastase at 37 C for 2 hr, then osmication and embedding; (3) boiling of the formalin-fixed block in distilled water for 1 hr, followed by osmication and embedding.

Isolation of BD and Physicochemical Characterization

Five grams of fresh human myocardium obtained at necropsy and known to contain extensive BD were left to stand at 10 C for 30 hr to ensure a complete breakdown of the normal glycogen present.[17] After this, the Pflüger's method for glycogen isolation was employed.[18] The same procedure was applied to myocardium from 3 other cases in which BD was absent or negligible. The material isolated from the former case was studied for (1) determination of the amount of glucose present after acid hydrolysis with the enzymatic cycling method of Lowry *et al* [19]; (2) determination of the degree of degradation obtained by the combined action of phosphorylase and debranching enzyme (amylo-1,6-glucosidase) [20]; (3) measurement of the infrared spectrum with a Perkin-Elmer model 237B grating infrared spectrophotometer,[21] using as reference compound normal glycogen (extracted from rabbit liver).

Results

Light Microscopic Appearance

BD was localized in the center of the cytoplasm of the myocardial fibers, surrounding the nucleus and along the greater axis of the cell. It was blue with hematoxylin-eosin (Fig 1a) (hence the term "basophilic") and strongly PAS-positive. In the slightly affected fibers, it appeared like a fine granular deposition in or between the myofibrils (Fig 1b); in

more advanced stages, the granules joined together to form longitudinal striae or dense masses (Fig 1c). Occasionally the deposits of BD had an altogether different disposition, forming well-delimited, crystal-like, homogeneous masses of sharp edges, which stained lighter with PAS (Fig 1d). Myofibrils were present in the area of BD in some cells and absent in others. Even in the most affected fibers, a thin peripheral layer of cytoplasm with preserved myofibrils could be seen. The nucleus was usually deformed by compression, but otherwise unaltered. Only occasionally it was picnotic. The fibers affected with BD were otherwise unaltered, and the surrounding tissues lacked any sign of reaction to the presence of this abnormal substance. Characteristically, only isolated fibers were involved. There was no relation between the amount of BD and that of lipofuscin pigment or with the trophic state of the myocardial fibers.

Frequency and Extent

The frequency and extent of BD in our material is shown in Table 1. BD was not seen in any of the patients who had died in the first decade. Conversely, it was present in all the individuals after the age of 11 years, with only one exception. The overall incidence was 88.88%. Four arbitrary grades were used to quantitate the extent of BD. In Grade 1, only one or two affected fibers were found in the whole section; in Grade 4, several involved fibers were seen in every low-power field. Grades 2 and 3 were intermediate. In the least affected cases, high-power examination of PAS-stained sections was necessary to discover

Table 1. Frequency and Extent of Basophilic Degeneration

Age group (in decades)	No. of affected myocardial fibers (expressed in grades)					Total No. of cases
	Negative	1	2	3	4	
0–10	14	—	—	—	—	14
11–20	—	3	1	—	—	4
21–30	1	6	1	—	—	8
31–40	—	10	1	—	—	11
41–50	—	12	2	—	—	14
51–60	—	15	8	2	—	25
61–70	—	20	7	2	1	30
71–80	—	10	7	3	3	23
81–90	—	3	1	1	—	5
91–100	—	—	1	—	—	1
Total		79(66%)	29(24%)	8(7%)	4(3%)	135

slightly degenerated fibers. Of a total of 120 positive cases, Grade 1 was found in 66%; Grade 2 in 24%; Grade 3 in 7%; and Grade 4 in 3%.

Histochemical Features

The staining reactions of BD are summarized in Table 2 (Fig 1a to 1i). The influence of various treatments on its PAS-positivity and slight Alcian blue (pH 2.5)-positivity is shown in Tables 3 and 4, respectively (Fig 1j to 1o).

Fine Structural Morphology and Histochemistry

The deposits of BD were seen as sharply circumscribed, homogeneous areas of low electron density, located in the middle of the myocardial fiber, usually centered by a deformed nucleus (Fig 2). No limiting membrane was present between the deposits and the surrounding myofibrils. The predominant component of BD consisted of short, straight fibrils, approximately 60–70 Å in width, running in a haphazard fashion but occasionally paired, with no axial periodicity (Fig 3). Scattered among the fibrils were a few round, dense granules, 150 Å in diameter, compatible with glycogen β particles; scattered mitochondria; short fragments of myofibrils; isolated myelin figures; a few lysosomes, some containing lipofuscin pigment; and disperse small granules of higher electron density than the fibrils. No morphologic abnormalities of the organelles of the affected fibers were detected.

After diastase digestion, done either in the blocks or in the sections, the fibrils had largely disappeared, leaving in its place an extremely fine granular material of very low electron density (Fig 4).

Boiling of the tissue block in distilled water also led to disintegration of the fibrils into a granular material, this one coarser and more electron-dense than that observed after diastase digestion (Fig 5).

Table 2. Histochemical Reactions of Basophilic Degeneration

Stain	Reaction	Stain	Reaction
Hematoxylin-eosin	Blue ("basophilic")	Sudan IV	Negative
Periodic acid-Schiff	Positive	von Kossa	Negative
Langhans' iodine	Positive	Verhoeff's elastin	Negative
Best's carmine	Positive	Mayer's mucicarmine	Negative
Gomori's lithium-silver	Positive	Lillie's azur A-eosin B	Negative
Feulgen reaction	Negative	Toluidine blue	Negative
Mallory's PTAH	Negative	Alcian blue (pH 2.5)	Slightly positive
Bennhold's Congo red	Negative	Alcian blue (pH 1.0)	Negative
Crystal violet	Negative	Mowry's colloidal iron	Positive
Thioflavine T	Negative	Millon reaction	Negative
Perls'	Negative	Autofluorescence	Negative

Table 3. Effect of Various Treatments on the PAS-Positivity of Basophilic Degeneration and Related Substances

Treatment	BD	Material of glycogenosis IV*	Glycogen†	Mucosubstances‡
Diastase, 30 min	Practically unchanged	Slightly decreased	Abolished	Unchanged
Diastase, 8 hr	Moderately decreased	Moderately decreased	Abolished	Unchanged
Diastase, 48 hr	Practically abolished	Practically abolished	Abolished	Unchanged
Chloral hydrate + diastase, 30 min	Abolished	Abolished	Abolished	Unchanged
Boiling + diastase, 30 min	Abolished	Abolished	Abolished	Unchanged
Amyloglucosidase	Abolished	Abolished	Abolished	Unchanged
Pectinase	Abolished	Abolished	Abolished	Unchanged
Trypsin	Unchanged	Unchanged	Unchanged	Unchanged
Xylene + chloroform + ether	Unchanged	Unchanged	Unchanged	Unchanged

* From myocardium. † From liver. ‡ From stomach, large bowel, uterine cervix.

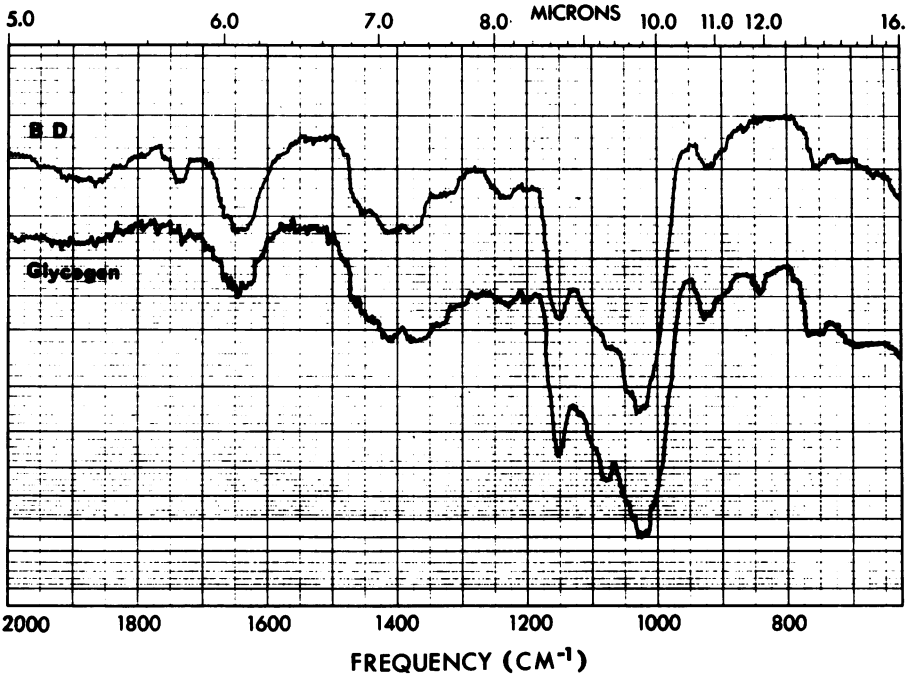
Physicochemical Characterization

The material isolated from myocardium with extensive BD was found to have a concentration of 0.92 μmoles of polyglucose/g, whereas that isolated from the 3 control cases had virtually none. The former was completely degraded by the combined action of phophorylase and debranching enzyme, the ratio of glucose to glucose + glucose-1-phosphate being 11.4%. The infrared spectrum of isolated BD was practically identical to that of a sample of rabbit liver glycogen, with only a minor difference; instead of the sharp absorption peak at about 11.85 μ present in glycogen, BD had two, which were barely detectable, at 11.6 and 11.85 μ (Text-fig 1).

Table 4. Effect of Various Treatments on the Slight Alcian Blue (pH 2.5) Positivity of Basophilic Degeneration and Related Substances

Treatment	BD	Material of glycogenosis IV*	Glycogen†	Mucosubstances‡
Diastase, 30 min	Practically abolished	Practically abolished	—	Unchanged
Chloral hydrate	Abolished	Abolished	—	Unchanged
Boiling	Abolished	Abolished	—	Unchanged
Amyloglucosidase	Abolished	Abolished	—	Unchanged

* From myocardium. †From liver. ‡ From stomach, large bowel, uterine cervix.



TEXT-FIG 1. Infrared spectra of BD and normal glycogen extracted from rabbit liver, the latter used for comparison.

Discussion

Several opinions have been expressed in the literature about the chemical nature of BD, all based on the interpretation of histochemical reactions performed at a light microscopic level. According to some, it represents nuclear materal (deoxyribonucleic acid, ribonucleic acid, or both) dispersed in the cytoplasm.[1,11] Others believe it is a glycogen-protein complex,[9,14] perhaps with a molecular abnormality of the glycogen component.[14] Most workers consider BD to be of "mucinous" or "mucoid" nature (either a muco/glycoprotein or an acid mucopolysaccharide).[2-7,12]

The possibility that BD contains nucleic acids can be discarded on several grounds. BD is negative with the Feulgen reaction for DNA and with Lillie's azur A-eosin B for RNA; it is positive with PAS, Best's carmine and Langhans' iodine (none of which stains nucleic acids); and is unaffected by the action of ribonuclease.[4] The statement that BD is a mucosubstance has been based on the alleged positivity of the Mayer's mucicarmine reaction, the toluidine blue metachromasia and the resistance to the action of diastase, three features which would seem to exclude glycogen or a related glucose polymer (glucan). On the other

hand, the positivity of BD with Best's carmine and Langhans' iodine is more suggestive of glycogen than it is of a mucosubstance. Unfortunately, none of these stains is absolutely specific,[22] and therefore no definite conclusions could be made from their use alone.

The results we have obtained with this combined histochemical, ultrastructural and biochemical approach favor the interpretation that the material of BD is basically composed of a glucan (polyglucosan). While unaffected by hyaluronidase or trypsin, it was completely degraded by amyloglucosidase, an enzyme with specific hydrolytic activity on α-1, 4- and α-1,6-glucan links.[23] Although seemingly resistant to malt diastase if this was applied for the conventional 30-min period, it was completely degraded if the treatment was prolonged, as shown by both light and electron microscopy. Malt diastase is mainly composed of α-amylase, with a smaller amount of β-amylase, two enzymes with a specific hydrolytic action on the α-1,4-glucan links in polysaccharides.[23] Furthermore, the boiling of the tissue before embedding or the treatment of the sections with chloral hydrate rendered the material of BD as susceptible to diastase as normal glycogen, whereas it did not affect the mucosubstances tested as controls. This behavior of BD towards diastase after boiling and chloral hydrate is identical to that observed with other glucans, such as the abnormal glycogen of glycogenosis IV,[24] and the corpora amylacea of central nervous system.[25] It has been suggested that the relative resistance of these substances to amylase digestion is due to intermolecular hydrogen bonding, and that the boiling or the chloral hydrate disrupts these bonds.[24] Electron microscopic examination of BD after boiling shows indeed a complete breakdown of the filamentous material. Alternatively, the lesser effectiveness of amylase over amyloglucosidase could be due to the presence of a large number of branching points in the BD molecule. As mentioned above, 1,6 links are unaffected by amylase but are hydrolyzed by amyloglucosidase.

The biochemical results with the isolated material further substantiate the glucan composition of BD. The complete degradation by phosphorylase and debranching enzyme indicates that α-glucose is the basic, and probably the exclusive monosaccharide present. If this is the case, the possibility that BD is composed of an acid mucopolysaccaride or a glyco/mucoprotein can be discarded, since glucose is never a constituent of the former,[26] and only exceptionally of the latter, where it represents only a small portion of the moiety.[27] The 11.4% ratio of glucose to glucose + glucose-1-phosphate suggests a high degree of branching, the ratio of normal glycogen being 7–8%. The infrared spectrum of BD is also characteristic of an α-D-glucan.[28] The absorption peaks at 10.8 and 13 μ confirm the presence of 1,4 links in the molecule. On the other hand,

the small difference between BD and the usual liver glycogen in regard to the peak at 11.85 μ and the appearance of a second at 11.6 μ suggests that isolated BD contains little in the way of branching points and that the predominant linkage is of the α-1,4 type.[21]

Once it is established that BD is a polysaccharide formed by α-D-glucose units, the logical assumption is that it represents some type of glycogen, since this is the only glucan normally found in vertebrate tissues. However, BD differs from glycogen in several respects, such as electron microscopic appearance, relative resistance to diastase digestion and acidic properties. Fibrils and finely granular material, for instance, do not correspond to any of the previously described fine structural appearances of normal mammalian glycogen. To help understand these apparent inconsistencies, it is pertinent to compare the features of BD with those of three other substances that can be found in human tissues. These are (1) the round, amorphous, basophilic bodies located inside the processes of fibrous astrocytes and known as *corpora amylacea,* which are practically always present in otherwise normal senile brains and are abundant in some cases of epilepsy; (2) the intraneuronal round structures known as *Lafora bodies,* present in the familial form of myoclonus epilepsy (Lafora's disease) [21]; and (3) the generalized deposits of abnormal glycogen in *Type IV glycogenosis.*[24]

These three substances share with BD the intracytoplasmic location, basophilia, histochemical reactions, and behavior towards enzymatic digestions. Ultrastructurally, they all have a basic fibrillary component, with the admixture of a few scattered glycogen particles.[24,29,30] After performing on corpora amylacea the same histochemical reactions that we have now applied to BD, one of us (JR) postulated the glucan nature of the former.[25] This has been confirmed by Sakai *et al,*[31] who after isolating and analyzing corpora amylacea by enzymatic digestion, chromatography and infrared spectrophotometry, found them to be polymers of α-glucose instead of acid mucopolysaccharides. Identical conclusions have been reached by Yokoi *et al*[21] after applying similar technics to isolated Lafora bodies. In both instances, it was suggested that these deposits represented a disorder in carbohydrate and specifically glycogen metabolism. A point which strongly favors this interpretation is that in Type IV glycogenosis, a disease resulting from a deficiency of an enzyme involved in glycogen synthesis (branching enzyme, α-1,4-glucan:α-1,4-glucan 6-glycosyltransferase), the abnormal, amylopectin-like glycogen, which is deposited as a result, has very similar features, as the comparative studies shown in this report and those performed by Reed [24] indicate.

In the light of these observations, we postulate that the branched glucan deposited in BD represents a relatively insoluble by-product of glycogen metabolism, possibly brought upon by an acquired relative deficiency of an enzyme (or enzymes) of the glycogenesis-glycogenolysis pathway. It is likely that the branching pattern of BD is different from that of normal glycogen, and that this difference is the main reason for its changed properties. The information we have obtained is, however, contradictory in this regard. The marked similarity to the material of glycogenosis IV plus the characteristics of the infrared spectrum would suggest that the glucan of BD has long outer chains and relatively few branching points. On the other hand, the greater effectiveness of amyloglucosidase (which acts both on α-1,4 and α-1,6 linkages) over amylase (which acts only on α-1,4 linkages), and the results of the enzymatic digestion of the isolated material would indicate a high degree of branching.

The fact that the material of glycogenosis IV is fibrillary when examined under the electron microscope, indicates that glucose polymers can depart from the rosette appearance of normal glycogen. It is interesting in this regard that Becker *et al*[32] have noted a fibril-like structure when examining aggregates of different glucans *in vitro*.

The reason for the acidic properties of BD, which are shared by glycogenosis IV, corpora amylacea and Lafora bodies, is not entirely clear. The results outlined in Table 4 suggest that these properties are located in the glucose units. Two possibilities should be considered: (1) the stains employed are not actually demonstrating acid groups but rather another type of reaction inherent to the glucan molecule[24]; (2) in addition to glucan, other chemical groups (*ie*, phosphate) are present, either esterified in the glucan *per se*[31] or passively enmeshed among glucan aggregates.

What is the significance of BD? It is primarily a human condition, although it has been seen exceptionally in animals.[33] It is not a postmortem phenomenon, since it has been seen in the myocardium of auricular appendages removed at surgery.[34] No clinical symptoms have been attributed to its occurrence, a fact hardly surprising in view of the fact that even in the more severe cases only a small percentage of the fibers are affected. As our survey indicates, BD is mainly related with the age of the individual, and is as common an occurrence as corpora amylacea of the central nervous system. In the following three diseases, however, BD is usually present in a much larger amount that what one would expect from age alone.

1. *Lafora's disease*, a familial form of myoclonic epilepsy.[21,35,36] This

is particularly interesting in view of the fact that the intraneuronal Lafora's bodies and the systemic deposits present in this disease have, as previously mentioned, the same biochemical, histochemical and ultrastructural properties of BD. Since it has been suggested that the material deposited in the heart in patients with Lafora's disease is histochemically different from that of BD,[25] we examined one such case with the histochemical technics we used for the other cases of BD. We could detect no differences between the two groups.

2. *Hypothyroidism.* The presence of BD in the heart of hypothyroid patients is so constant and striking that it has been said that "the great increase in their number constitutes the histologic characteristic of the myxedema heart."[37] This should not be surprising, since it is well known that thyroid hormone regulates the metabolism of cardiac glycogen by heightening the glycogenolytic effect of catecholamines [38] and perhaps also through a direct action on the enzymes involved in glycogen synthesis and degradation.[39] Although BD has not been produced experimentally, it is known that thyroidectomy increases the content of cardiac glycogen in experimental animals.[38] We found no histochemical or ultrastructural differences between the cases of BD seen in euthyroid individuals as compared with myxedematous patients, although we did not perform the phosphorylation procedures described by Haust *et al.*[5]

3. *Idiopathic myocardiopathy.* Patients with this condition have sometimes been found to have extensive BD in their myocardial fibers.[40] It is uncertain if BD plays some part in the pathogenesis of this disease or if it is merely a nonrelated finding.

The presence of BD in these three conditions indicates that the deposition of this substance is not always a simple epiphenomenon of aging. Its occurrence in Lafora's disease, hypothyroidism, idiopathic myocardiopathy, as well as in aging, should be viewed rather as a morphologic expression of a disturbance in the glycogen metabolism of the myocardial fibers.

Summary

The name basophilic (mucoid) degeneration of myocardium is applied to a common disorder of the myocardial fibers characterized by the deposition of a granular, dense or crystal-like substance in their cytoplasm. In the present study it was found in 88.88% of the hearts examined. There was a definite correlation with the age of the individual affected. BD was positive with all of the glycogen stains employed. It was digested by amyloglucosidase, malt diastase and pectinase. Ultrastructurally, it was characterized by a conglomerate of 60–70 Å fibrils

admixed with a few particles consistent with glycogen β particles. The isolated material was a glucose polymer, and was totally degraded by the combined action of phosphorylase and debranching enzyme. Its infrared spectrum was practically identical to that of glycogen. On the basis of these findings and its correlation with other abnormal deposits (Lafora's bodies, corpora amylacea and the material of glycogenosis IV), it is postulated that BD is made of a glucan and that it represents a by-product of glycogen metabolism, possibly brought upon by an enzymatic deficiency of the glycogenesis-glycogenolysis pathway.

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[*Illustrations follow*]

Legends for Figures

Fig 1. Light microscopic appearance and histochemical reaction of BD. × 150. (**a**) Hematoxylin-eosin. Finely granular material of low density present in the center of a myocardial fiber, producing compression of the two nuclei. (**b**) PAS. Fine linear deposits following the direction of the myofibrils. (**c**) PAS. Large clump of dense material, with a thin rim of noninvolved cytoplasm. (**d**) PAS. Homogeneous, crystal-like disposition of BD. Lipofuscin granules are also present. (**e**) Langhans' iodine. (**f**) Best's carmine. (**g**) Gomori's lithium-silver. (**h**) Alcian Blue (pH 2.5). (**i**) Colloidal iron. (**j**) Diastase, 30 min; PAS. There is practically no change. (**k**) Diastase, 8 hr; PAS. The deposits of BD are barely recognizable *(arrows)*. (**l**) Diastase, 48 hr; PAS. BD has completely disappeared, leaving an empty space in the cytoplasm *(arrows)*. (**m**) Chloral hydrate-diastase, 30 min; PAS. The material of BD has been totally digested *(arrows)*. (**n**) Boiling-diastase, 30 min; PAS. Total disappearance of BD. (**o**) Amyloglucosidase-PAS. Total disappearance of BD.

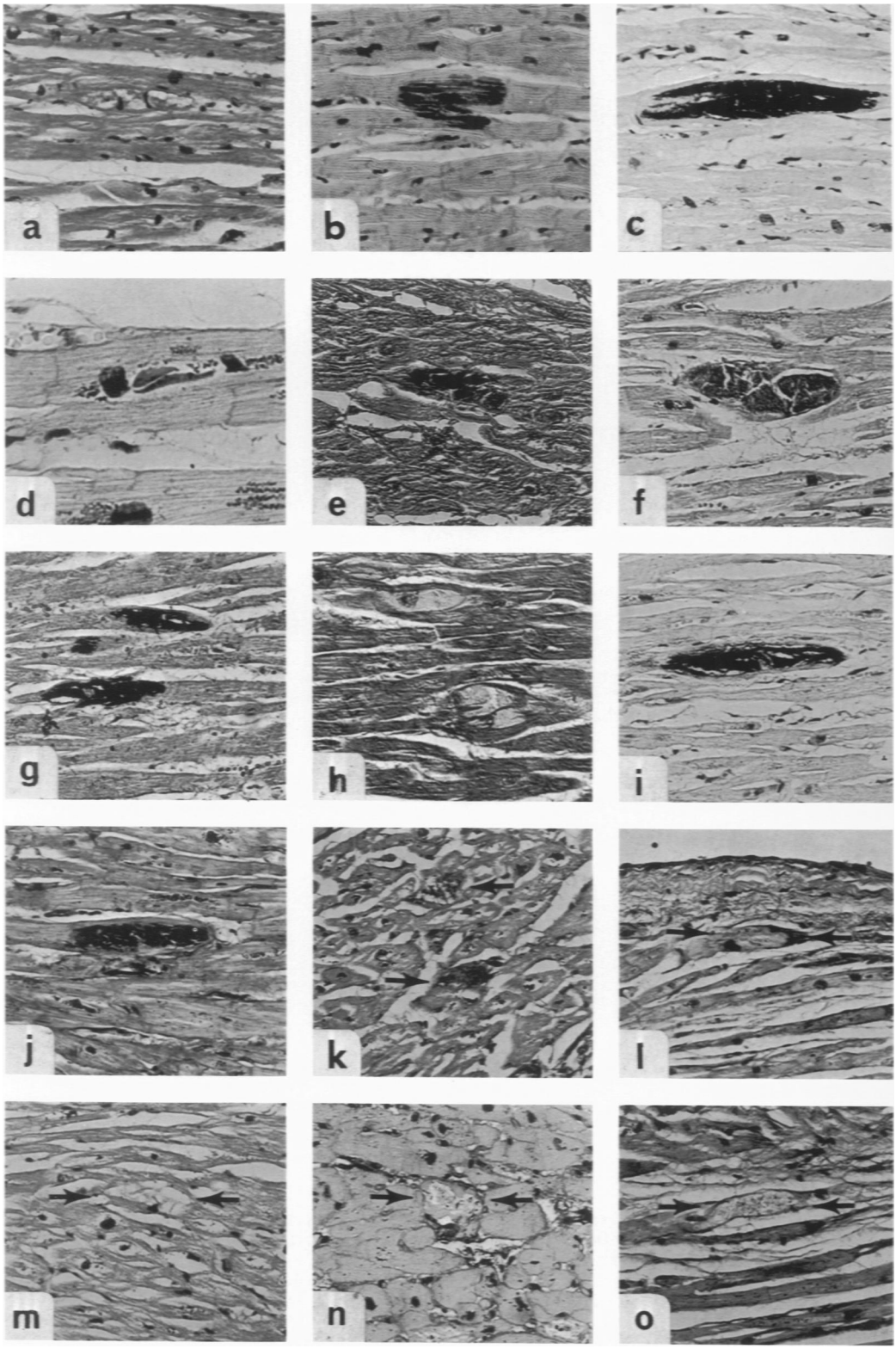
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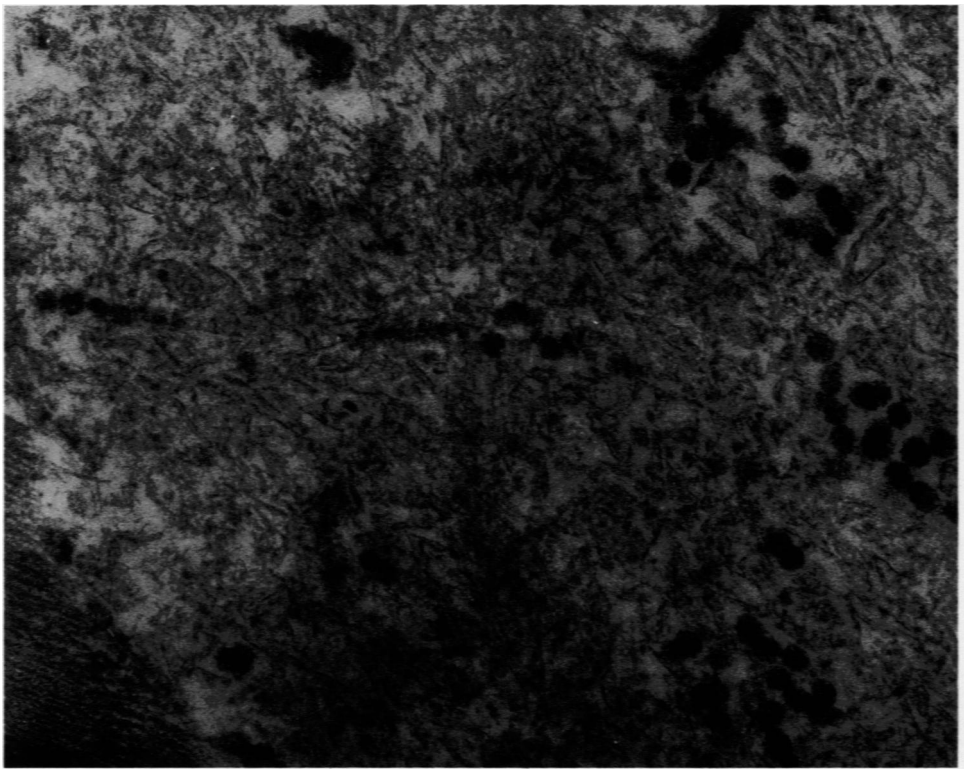
Fig 2. Low-power electron micrograph of BD. Homogeneous material of low electron density is seen in center of myocardial fiber, compressing nucleus and leaving rim of myofibrils at periphery. Uranyl acetate-lead citrate. × 2085.

Fig 3. High-power electron micrograph of BD. Principal component is a filamentous material randomly distributed. Scattered round structures consistent with glycogen particles are also present. Uranyl acetate-lead citrate. × 40,350.

Fig 4. Fine structural appearance of BD after digestion of thin section by diastase. Both filaments and round structures have largely disappeared. Uranyl acetate-lead citrate. × 19,500.



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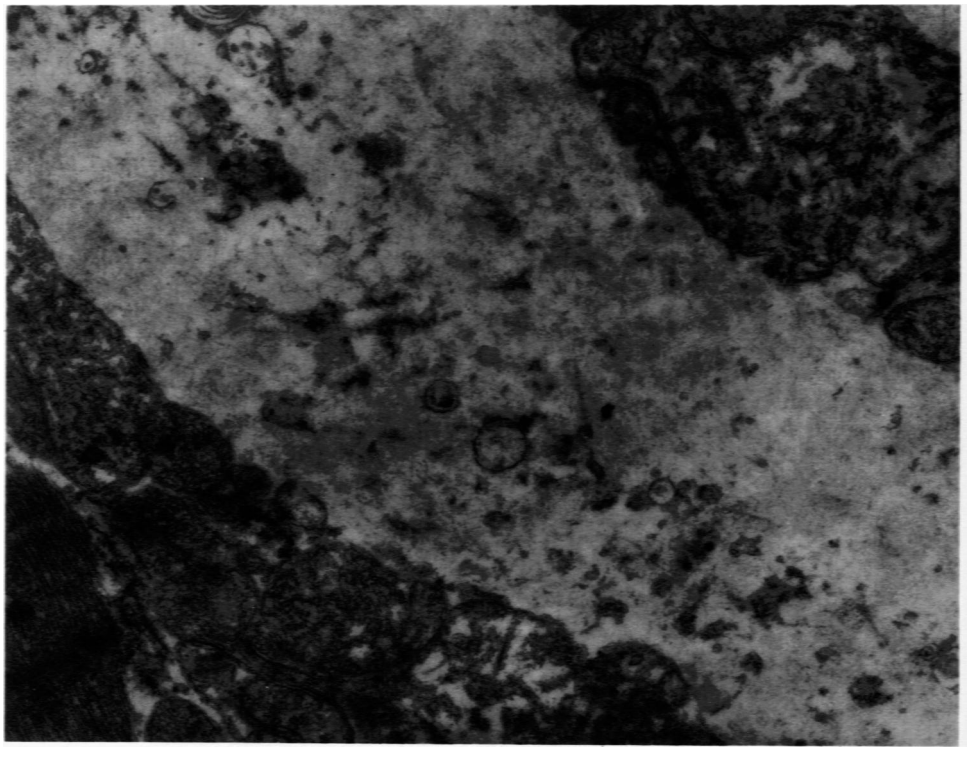

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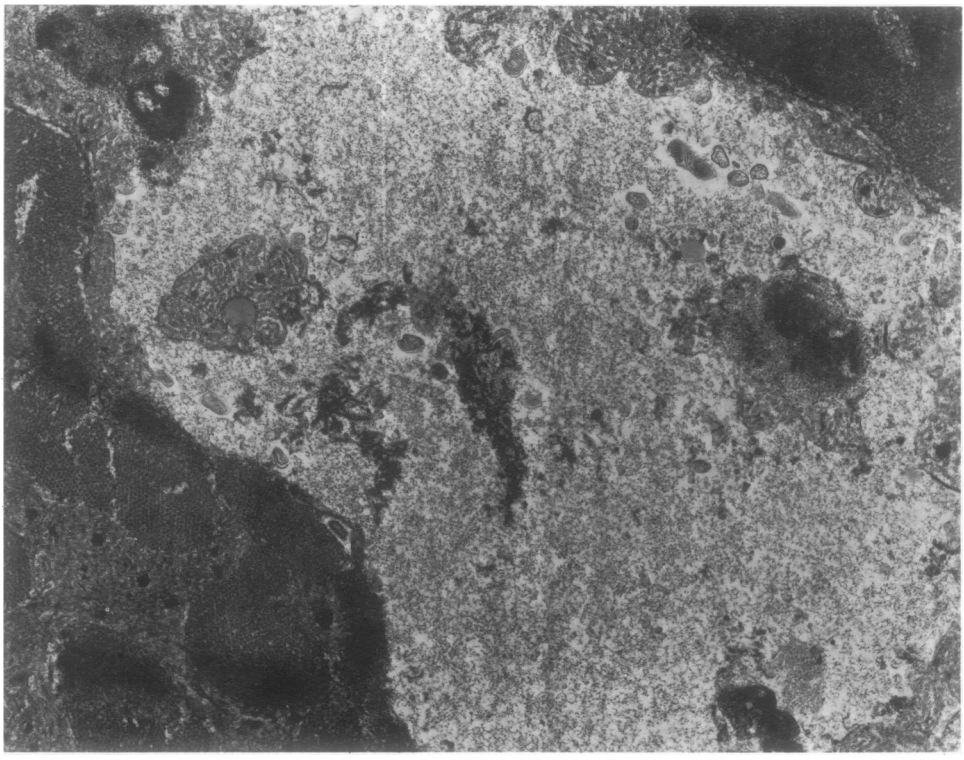

Fig 5. Fine structural appearance of BD after boiling of formalin-fixed block. Filaments have broken down into finely granular material of high density. Uranyl acetate-lead citrate. × 13,650.